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US 4382921 A
EMBASE Acc.No.84174787 & G.Ital.Dermatol.
Venereol.(Italy) 119/2, page 129, (1984) EMBASE
Acc.No.84025726 & Dermatol. Monatsschr.
(E.Germany) 169/9, pages 581-7, (1983) EMBASE
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(1982) EMBASE Acc.No.82029850 & Arch.Dermatol.
Res.(W.Germany), 271/4, pages 437-9, (1981)

(58) continued overleaf

(54) Treatment of psoriasis

(57) The use in the preparation of a pharmaceutical formulation for the treatment of psoriasis of N-desmethyl tamoxifen, 4-hydroxy tamoxifen, N-desmethyl droloxifene, 4-hydroxy droloxifene, miconazole, bromocriptine, flunarizine, dequalinium, or a pharmaceutically acceptable salt thereof.

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(58) Field of Search
UK CL (Edition L) A5B BHA BJA
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CAS-ONLINE

PHARMACEUTICAL PREPARATION FOR THE TREATMENT OF PSORIASIS

This invention relates to the use in the treatment of psoriasis of certain pharmaceutically active agents.

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It has been known for some years that a connection exists between dermatological conditions such as psoriasis, and the level of calmodulin present in the epidermis of the affected patient. The following references are exemplary of studies in which this connection has been investigated.

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- S. Mac Neil et al., Clinical Science (1985); 69, 681-686;
W.F.G. Tucker et al., Acta Derm Venerol (Stockh) 1986; 66:241-244;
W.F.G. Tucker et al., J Invest Dermatol 87:232-235, 1986;
15 A.M. Al-Ani et al., British Journal of Dermatology (1988) 119, 295-306;
W.F.G. Tucker et al., Journal of Investigative Dermatology, 82:298-299, 1984.

15

It has also been demonstrated that certain materials know to have calmodulin antagonist activity are effective in the therapeutical treatment of psoriasis.

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We have now discovered that miconazole, droloxifene, bromocriptine, flunarizine and degualinium and their pharmaceutically acceptable salts, which are known to have pharmaceutical activity for other purposes but have not previously been suggested as being suitable in the treatment of psoriasis, are effective as calmodulin antagonists both *in vitro* and *in vivo*, and are of therapeutic value in the treatment of psoriasis.

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We have also discovered certain metabolites of tamoxifen and droloxifene not previously known as calmodulin antagonists (namely N-desmethyl tamoxifen, 4-hydroxy tamoxifen, N-desmethyl droloxifen, and 4-hydroxy droloxifen), have significant calmodulin antagonist activity and are of therapeutical value in the treatment of

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psoriasis.

The present invention provides the use of miconazole, droloxifene, bromocriptine, flunarizine, dequalinium, N-desmethyl tamoxifen, 4-hydroxy tamoxifen, N-desmethyl droloxifene, or 4-hydroxy droloxifene, or their pharmaceutically acceptable salts, as an active agent in the preparation of a pharmaceutical formulation for the treatment of psoriasis.

As noted above, dequalinium, droloxifene, bromocriptine, flunarizine, and miconazole, are known clinically for other indications, but none has previously been reported to be useful in the treatment of psoriasis. Flunarizine is employed as a vasodilator, and is employed systemically, bromocriptine is known as a dopamine receptor antagonist, again for systemic use, miconazole is used as an anti-fungal agent, droloxifene as an anti-tumour agent, and dequalinium salts, particularly dequalinium chloride, are employed topically as antimicrobial agents.

Any of the pharmaceutically acceptable salts of the active materials noted above may be employed in the present invention.

Suitably pharmaceutically acceptable salts are hydrochloride, hydrobromide, citrate, and D-gluconate.

Since we have observed calmodulin antagonist activity in metabolites of tamoxifen and droloxifene as noted above, metabolites of dequalinium, bromocriptine, flunarizine, and miconazole, may also possess such activity, and accordingly the invention includes within its scope the use of a pharmaceutically acceptable metabolite of one of the said active agents, which itself has calmodulin antagonist activity.

The invention is illustrated in the following examples:

Assay for Calmodulin Activity

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The active agents were assayed for calmodulin antagonist activity by the method described by Mac Neil et al (Biochem Pharmacol 1988; 37:1717-23). Each of the drugs examined were investigated in a minimum of three assays, and results calculated as mean \pm SEM of IC₅₀ values.

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Results are shown in Table 1. Also indicated in Table 1 are the calmodulin antagonist activity, when assayed by the same method, of tamoxifen, chlorpromazine and dithranol, which have previously been proposed for use in the treatment of psoriasis.

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Determination of Calmodulin Antagonist Activity by Cell Proliferation Measurement

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Skin was obtained from abdoninoplasties, breast reductions and circumcisions. The subcutaneous fat was removed, the skin cut into 0.5 cm squares and incubated in 0.1 percent trypsin, 0.1 percent glucose and 0.01 percent phenol red at pH 7.4 at 4°C for eighteen hours. Skin pieces were then washed in phosphate buffered saline (PBS), dermal and epidermal layers separated, and keratinocytes collected into PBS plus 10 percent foetal calf serum (FCS) from both the underside of the epidermis and the upper surface of the dermis by scraping with a scalpel blade. Cells were washed, resuspended in medium, and cell number and viability determined.

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The two media used were both serum free; medium 199 supplied by Northumbria Biologicals (physiological calcium), and MCDB153 supplied by Sigma Chemical Co. (low

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calcium). Both media contained specified mitogens and bovine pituitary extract. Medium 199 with 2mM glutamine and 22g/l sodium bicarbonate, and MCDB153 (made up according to accompanying instructions), were each
5 supplemented with penicillin (100 units/ml), 100 µg/ml streptomycin, 0.63 µg/ml Fungizone®, 1 percent bovine pituitary extract, 10µg/ml EGF, 2.5 µg/ml hydrocortisone, 10 µg/ml insulin and 0.1 µg/ml phosphoethanolamine.

10 Approximately 2×10^5 cells/ml/well (3.5 cm in diameter) were plated out and allowed to attach for 48 hours. The cells were then washed, and fresh medium containing the drugs to be examined was added for a further 45 hours. Cells were cultured in a CO₂-gassed incubator.
15 All experiments were serum-free unless indicated otherwise.

Determination of Cell Number

Cell number was determined in one of three ways:-

- 20 (i) directly, by trypsinizing cells and then using a haemocytometer,
(ii) directly by counting the number of cells per mm² of tissue-culture dish using an eye-piece graticule, and
(iii) indirectly, using the MTT-ESTA assay.

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The basis of the MTT-ESTA assay is that the MTT (3-(4,5)-dimethylthiazol-2-yl-2,5-phenyltetrazolium bromide) acts as an artificial hydrogen-acceptor substrate for dehydrogenase activity in the cell. The reduced MTT
30 forms a coloured formazan product which is then eluted from the cells using acidified isopropanol. This cyto-biochemical assay can provide an indirect reflection of cell number, in that dehydrogenase activity usually relates to cell number. In practice, the effects of drugs on cell
35 number were always visually assessed prior to termination of the experiment with the MTT-ESTA assay, and throughout

this study dehydrogenase activity reflected the changes in cell number seen by eye.

Experiments were terminated after 45 hours of drug incubation by removal of media, and cells were washed with PBS. They were then incubated with 1 ml of 0.5 mg/ml MTT in PBS for 40 minutes at 37°C in a CO₂-gassed incubator. The MTT was then removed, and the dye which had been incorporated into the cells and converted into a coloured formazan product was then eluted by addition of 300 µl of acidified isopropanol (25 µl of concentrated HCl to 20 ml of isopropanol). The optical density of the eluted MTT product was measured at a test wavelength of 570 nm with a reference wavelength of 630nm to account for varying amounts of cell protein.

Drugs were made up as 10mM stock solutions in 100 percent DMSO. Control incubations containing equivalent concentrations of DMSO were performed in parallel. DMSO produced approximately 10 percent inhibition of keratinocyte proliferation at a concentration of 3 percent. The active agents with which the present invention is concerned inhibited keratinocyte proliferation completely by 100 µm.

Drugs were examined in cell growth in both Medium 199 (physiological calcium) and MDCB153 (low calcium) in a minimum of two experiments in each medium. Triplicate cultures of wells were used for each concentration of drug. Results are expressed as means ± SEM of the concentrations required to produce 50 percent inhibition (IC₅₀).

The results are also shown in Table 1. Table 1 also compares the inhibitory potency of the active agents as antiproliferative agents and as calmodulin antagonists. Analysis of the antiproliferative activity in cells grown

in both media M1999 and MCDB153 revealed no significant difference; hence data for the two media were combined to give the results shown in Table 1.

5 The active agents used in accordance with the invention may be formulated in any convenient topical vehicle for use. Conveniently, the vehicle may comprise a hydrophilic cream or lotion, to which an aqueous solution or suspension of the active agent is added.

10 Alternatively, the vehicle may be in the form of a bath oil, although any other compatible topical vehicle can be used to provide the topical vehicle. The active agents will generally be present in the topical vehicle in a concentration of from 0.1 to 5 percent by weight.

Table 1

Drug	IC ₅₀ (μM)	
	Cell Proliferation	Calmodulin Activity
Bromocriptine	15±2	7±2
Flunarizine	5±1	3±3.0
Dequalinium Chloride	4±0.6	6±1.2
Miconazole	5±1	8±3.0
N-desmethyl tamoxifen	-	1.9±0.5
4-hydroxy tamoxifen	-	1.9±0.4
N-desmethyl droloxifene	-	2.4±0.5
4-hydroxy droloxifene	-	2.5±0.7
Droloxifene	-	3.6±0.8
Dithranol	-	24±2
Tamoxifen	13±3	1.9±0.3
Chlorpromazine	23±3	28±2.9

CLAIMS

1. The use in the preparation of a pharmaceutical
5 formulation for the treatment of psoriasis of
bromocriptine, flunarizine, dequalinium, droloxifene, N-
desmethyl droloxifene, 4-hydroxy droloxifene, N-desmethyl
tamoxifen, 4-hydroxy tamoxifen, miconazole, or a
10 pharmaceutically acceptable salt thereof.
2. The use as claimed in Claim 1, wherein the active
material is bromocriptine, flunarizine, dequalinium, or a
pharmaceutically acceptable salt thereof.
- 15 3. The use as claimed in Claim 1, wherein the active
material is flunarizine-chloride, bromocriptine mesylate,
or dequalinium chloride.
- 20 4. The use as claimed in any one of Claims 1 to 3, wherein
the pharmaceutical formulation is in the form of a topical
preparation.
5. The use as claimed in Claim 4, wherein the
pharmaceutical formulation contains the said active
25 material in an amount of 0.1 to 5 percent by weight.
6. The use as claimed in any Claim 5 or Claim 6, wherein
the pharmaceutical formulation is in the form of a lotion,
a cream, or a bath oil.
- 30 7. A pharmaceutical preparation for the treatment of
psoriasis substantially as hereinbefore described.

**Examiner's report to the Comptroller under
Section 17 (The Search Report)** -8-

Application number

GB 9226832.5

Relevant Technical fields

(i) UK CI (Edition L) A5B (BHA, BJA)

(ii) Int CI (Edition 5) A61K 31/48

Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASES: DIALINDEX (MEDICINE), WPI,
CAS-ONLINE

Search Examiner

J F JENKINS

Date of Search

13 MAY 1993

Documents considered relevant following a search in respect of claims

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
X	US 4382921 (WEBER) See column 1 lines 52-53, column 2 lines 30-49 and Claim 3	1-3 and 5
X	Embase Acc Number 84174787 & G Ital Dermatol Venercol (Italy) 119/2, page 129 (1984) (LANDI et al)	1-6
X	Embase Acc Number 84025726 & Dermatol Monatsschr (E Germany) 169/9 pages 581-7 (1983) (WEBER et al)	1-6
X	Embase Acc Number 84018746 & Aktuel Dermatol (W Germany) 9/5, pages 172-4 (1983) (ALTMAYER et al)	1-6
X	Embase Acc Number 82160209 & Arch Dermatol Res (W Germany) 273/1-2 pages 159-60 (1982) (GUILHOU et al)	1-6
X	Embase Acc Number 82029850 & Arch Dermatol Res (W Germany) 271/4, pages 437-9 (1981) (WEBER et al)	1-6

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Categories of documents

X: Document indicating lack of novelty or of inventive step.

Y: Document indicating lack of inventive step if combined with one or more other documents of the same category.

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P: Document published on or after the declared priority date but before the filing date of the present application.

E: Patent document published on or after, but with priority date earlier than, the filing date of the present application.

&c: Member of the same patent family, corresponding document.

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